

CLAIMS:

1. A method for identifying a target of an antimicrobial compound comprising:
 - (a) cloning the open reading frame of a gene of a prokaryotic organism into an expression vector comprising an inducible promoter;
 - 5 (b) inducing expression of the gene with an inducer in the presence of an antimicrobial compound;
 - (c) comparing growth of cells from the induced gene expression in the vector to cells from the uninduced gene expression in the vector; and
 - (d) correlating the comparison to determine if the gene is resistant to the antimicrobial
10 compound and is a target of the compound.
2. The method of claim 1 wherein the growth of cells is compared using the minimum inhibitory concentration of the cells.
3. The method of claim 1 wherein the expression vector is a pYH4 vector having PmeI, NcoI, StuI, and AscI cloning sites.
- 15 4. The method of claim 3 wherein the expression vector further comprises a Pxyl/tet regulatory system, a ribosome binding site, and a transcriptional terminator.
5. The method of claim 1 wherein said prokaryotic organism is selected from the group consisting of *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*,
20 *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *alymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Kleibsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not

limited to, a member of the species or group, *Group A Streptococcus*, *Group B Streptococcus*, *Group C Streptococcus*, *Group D Streptococcus*, *Group G Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*,
5 *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium leprae*, *Actinomyces israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus*
10 *ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenteriae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii* and
15 *Chlamydia trachomatis*, (ii) an archaeon, including but not limited to *Archaeobacter*, and (iii) a unicellular or filamentous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus *Saccharomyces*, *Kluyveromyces*, or *Candida*, and a member of the species *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, or *Candida albicans*.

6. A method for detecting a target of an antimicrobial compound comprising:

20 (a) cloning substantially all of the genes of a prokaryotic organism's genome into expression vectors comprising an inducible promoter to form an ORF expression library;

(b) inducing expression of a copy of said library of genes with an inducer;

(c) contacting the copy of said library of induced genes and a copy of said library of uninduced genes with an antimicrobial compound, the antimicrobial compound killing the cells
25 with non-target genes; and

(d) determining the target gene of the antimicrobial compound by identifying the gene conferring cells survival of the treatment of the antimicrobial compound.

7. The method of claim 6 wherein the genes are fluorescence tagged to determine the identity of the gene conferring resistance to the antimicrobial compound.

8. The method of claim 6 wherein the genes and the antimicrobial compound are plated onto microtiter plates.

5 9. The method of claim 6 wherein the expressed, surviving gene is contacted with a DNA microarray to determine the identity of the surviving gene resistant to the antimicrobial compound.

10 10. The method of claim 6 wherein the genes are fluorescence tagged and contacted with a DNA microarray to determine the identity of the surviving gene resistant to the antimicrobial compound.

11. The method of claim 6 wherein the inducer is anhydrotetracycline.

12. The method of claim 6 wherein said prokaryotic organism is selected from the group consisting of *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*,
 15 *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *alymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Kleibsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not
 20 limited to, a member of the species or group, *Group A Streptococcus*, *Group B Streptococcus*, *Group C Streptococcus*, *Group D Streptococcus*, *Group G Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*,
 25 *Mycobacterium leprae*, *Actinomycetes israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordatella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus*

- ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenteriae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*,
- 5 *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii* and *Chlamydia trachomatis*, (ii) an archaeon, including but not limited to *Archaeobacter*, and (iii) a unicellular or filamentous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus *Saccharomyces*, *Kluyveromyces*, or *Candida*, and a member of the species *Saccharomyces cerevisiae*, *Kluyveromyces fragilis*, or *Candida albicans*.
- 10 13. A method of constructing a DNA library of substantially all genes of a prokaryotic organism comprising:
- (a) identifying the open reading frames of substantially all genes of a prokaryotic organism;
- (b) amplifying the DNA comprising the open reading frames; and
- 15 (c) cloning the open reading frames into expression vectors under control of an inducible promoter.
14. The method of claim 13 wherein the DNA is amplified using the polymerase chain reaction.
15. A method of constructing an expression vector comprising incorporating into a
- 20 DNA vector an inducible promoter system, a ribosome binding site, and multiple cloning sites, said cloning sites allowing the cloning of intact open reading frames of a prokaryotic organism.
16. The method of claim 15 wherein the DNA vector is a pYH4 vector and the cloning sites are PmeI and AscI sites.

17. The method of claim 15 further comprising incorporating into the DNA vector with replicons for *E. coli* and *S. aureus*.